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Phytochrome Destruction

AN APPARENT REQUIREMENT FOR PROTEIN SYNTHESIS IN THE INDUCTION OF THE DESTRUCTION MECHANISM[1]

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ABSTRACT

Examination of the phytochrome destruction reaction as a function of age in etiolated oat (*Avena sativa* L. cv. Garry) seedlings demonstrates that following illumination of 3-day-old shoots there is a lag, not observed in 4- or 5-day-old oats, prior to the onset of destruction. This light-mediated induction of the phytochrome destruction mechanism in 3-day-old shoots is inhibited by chloramphenicol, actinomycin D, and puromycin suggesting that protein synthesis is required. In 4-day-old shoots, actinomycin D and puromycin do not alter the kinetics of destruction while chloramphenicol partially inhibits the process. Thus, the inhibitors have a specific effect on the induction of the destruction mechanism but not its subsequent operation.

The photoreversible pigment phytochrome, which controls many facets of plant development, exists in a far red-absorbing, physiologically active form, Pfr, and a red-absorbing, physiologically inactive form, Pr. Upon photoconversion of Pr to Pfr *in vivo*, a rapid loss of spectrophotometrically detectable phytochrome ensues. Neither the mechanism nor the physiological significance, if any, of this destruction reaction are known.

Destruction generally follows first order kinetics with a half-life varying as a function of tissue age, degree of etiolation (9), and photostationary state (7). Butler and Lane (2) inhibited destruction by lowering oxygen concentration or by adding respiratory inhibitors such as carbon monoxide, cyanide, or azide. The destruction of Pfr is also repressed by metal-complexing and sulfhydryl compounds, while certain metallic ions such as zinc and iron partially reverse these types of inhibition (5, 8).

Pike and Briggs (11) reported destruction in rye tissue to be impeded by phenylmethylsulfonylfluoride and 2-mercaptoethanol but to be unaffected by dithionite. Finally, Bradley and Hillman (1) noted the insensitivity of phytochrome degradation *in vivo* to the respiratory uncoupler, 2,4-dinitrophenol. In sum, these data are consistent with the hypothesis that Pfr destruction requires metal ions and oxygen, not as a function of the respiratory chain or oxidative phosphorylation, but as a function of other reactions (1).

The present study examines destruction as a correlate of age in *Avena* seedlings and also shows the influence of puromycin, actinomycin D, and chloramphenicol upon both the appearance and operation of the mechanism responsible for the process.

MATERIALS AND METHODS

About 70 g of oat grains (*Avena sativa* L. cv. Garry) and 400 ml of distilled water were placed on one layer of cellulose packing material in 30 × 40 cm plastic cafeteria trays. The seedlings were used after 3 to 5 days at 25 ± 1 C at near saturating humidity in darkness with only occasional exposure to a dim green safelight.

Whole seedlings were totally immersed in unbuffered aqueous solutions of 1 mM chloramphenicol, 20 μg/ml actinomycin D, and 10 μg/ml puromycin aminonucleoside (all Sigma) and treated with two 2-min periods of vacuum infiltration using a Welch Duo Seal two stage pump and a 20-cm vacuum desiccator.

Actinic "red" irradiation was obtained from four closely spaced, unfiltered, 40-w Sylvania Gro-lux fluorescent tubes which were found to yield approximately the same photostationary state as 660 nm light. Far red irradiation was obtained from two 150-w reflector flood lamps filtered through 3.2 mm of far red Plexiglas (FRF-700, Westlake Plastic Co., Lenni, Pa.). Plant material was placed about 20 cm beneath either the far red or Gro-lux sources. Five minutes of red and 2 min of far red illumination were found to be saturating.

After the appropriate inhibitor application and light regime, whole shoots were harvested, minced with a razor blade, and gently packed into a chilled aluminum cuvette of 1 cm^2 cross-sectional area for measurement of total phytochrome (3, 4) in a custom built dual-wavelength difference spectrophotometer. Current from the photomultiplier (EMI 9558C) was passed through a Philbrick/Nexus logarithmic amplifier (No. 4351) and fed into an alternating current amplifier (Princeton Applied Research, Model 120) tuned to the frequency of the two alternating beams (90 hertz). Balzer B-20 interference filters (666 nm and 730 nm) were used to isolate the two measuring wavelengths from a 40-w quartz-iodine lamp powered by a Sorenson QRC 40-8A supply. The high voltage, supplied to the photomultiplier tube by a Kepco ABC 1000M supply, was always kept near 700 v, so that the measuring intensities could be as low as possible. The anode currents produced by the two measuring beams were approximately balanced using neutral density filters and maintained between 1 and 10 microamperes. Saturating actinic light was obtained by filtering the output of

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a Unitron Model LKR microscope lamp through either a Balzers B-40, 665 nm interference filter or 3.2 mm of Plexiglas (FRF-700). The measuring beams were reflected down through the vertical path cuvette by a partially reflecting mirror which allowed for actinic irradiation through the mirror without the necessity of moving the sample holder.

RESULTS

Table I indicates the average shoot length, average shoot weight, and initial $\Delta(\Delta A)$ values observed as a function of the plant ages used. As shown in experiments with oat seedlings of different ages using continuous illumination, the destruction mechanism was present and active immediately after conversion of Pr to Pfr in 4- or 5-day oats, but either was not present or was inactive for about the 1st hour after initial irradiation of 3-day seedlings (Fig. 1). This lag phase in 3-day oats was eliminated by pretreatment with 5 min Gro-lux, then 2 min far red, followed by a 90-min dark period and then re-exposure to 5 min Gro-lux illumination. Comparable seedlings exposed only to 5 min Gro-lux light demonstrated the lag phase (Fig. 2).

Three-day oats infiltrated with actinomycin D, chloramphenicol, or puromycin exhibited a prolongation of the lag phase with consequent inhibition of destruction following Gro-lux illumination (Figs. 3, 4, and 5). The controls, infiltrated with water prior to Gro-lux light exposure, demonstrated that the vacuum infiltration process affected neither the duration of the lag phase nor the rate of the destruction reaction (Figs. 3, 4, and 5). Four-day tissue, infiltrated with actinomycin D and puromycin immediately prior to illumination with red light (Figs. 4 and 5), displayed destruction kinetics comparable to those observed for 4-day tissue not treated with inhibitor (Fig. 1). However, the destruction reaction was partially inhibited in 4-day oats infiltrated with chloramphenicol (Fig. 3).

Table I. *Characterization of Etiolated Oat Shoots Used for Destruction Assays*

Seedling Age	Avg Shoot Length	Avg Weight/ Shoot	No. of Shoots Sample	Initial $\Delta(\Delta A)$/ Sample
days	*cm*	*mg*		
3	1.73	14	22	0.047
4	6.00	44	10	0.031
5	7.20	81	8	0.033

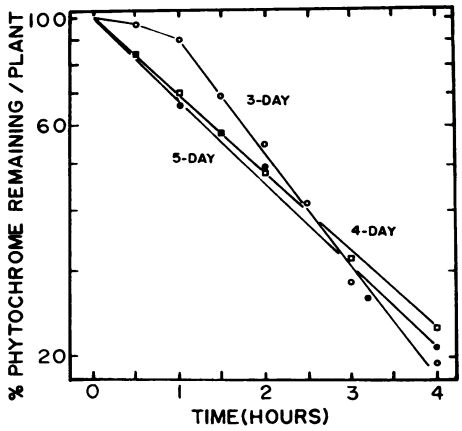

FIG. 1. Phytochrome destruction during continuous cool white fluorescent illumination in 3-day (○), 4-day (□) and 5-day (●) *Avena* seedlings. Each curve represents the average of four experimental replications.

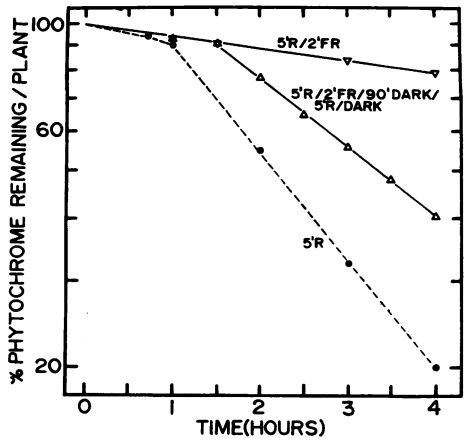

FIG. 2. Phytochrome destruction in 3-day Garry oats after various light treatments: 5 min Gro-lux followed by 2 min far red (▽); 5 min Gro-lux, 2 min far red, 90 min dark, 2 min Gro-lux (△); and 5 min Gro-lux (●). Each curve represents the average of four experimental replications.

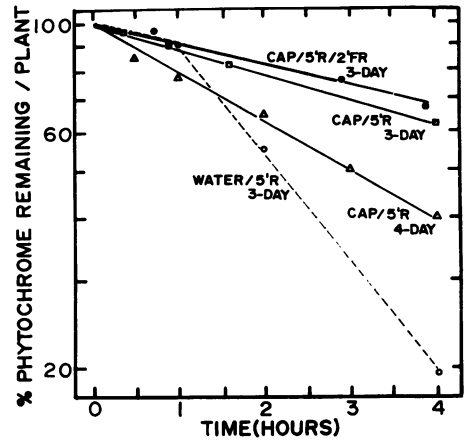

FIG. 3. Phytochrome destruction in (1) 3-day Garry oats vacuum infiltrated with 1 mM chloramphenicol followed by 5 min Gro-lux then 2 min far red light (●) or vacuum infiltrated with 1 mM chloramphenicol followed by 5 min Gro-lux (□) or vacuum infiltrated with water followed by 5 min Gro-lux light (○) and (2) 4-day seedlings vacuum infiltrated with 1 mM chloramphenicol followed by 5 min Gro-lux illumination (△). These curves represent the average of two experimental replications.

DISCUSSION

A lag phase prior to the onset of Pfr destruction, such as that seen in 3-day-old etiolated oats (Fig. 1), has also been reported in both *Pisum* (10) and *Sinapis* (9). Although the lag phase is eliminated in Garry oats by the 4th day following imbibition (Fig. 1), its elimination in younger tissue is clearly a light-induced response, although it is not a classical phytochrome-mediated response in that we were unable to reverse the Gro-lux light effect by far red irradiation (Fig. 2).

There are at least two possible interpretations for the presence of a lag phase prior to the onset of destruction. First, time might be required for the Pfr produced by the initial Gro-lux exposure to undergo a transformation to a form susceptible to destruction (9). Second, the lag period could represent the time required for a compound or structure to be synthesized in order for destruction to occur. The demonstration, by preirradiation with Gro-lux followed by far red light, that Pfr need not be present in order to eliminate the lag phase (Fig. 2) argues

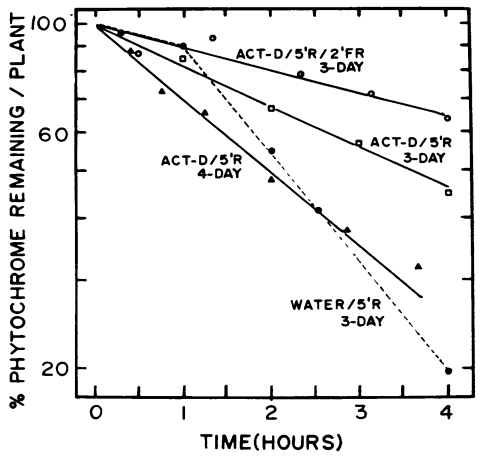

FIG. 4. Phytochrome destruction in (1) 3-day Garry oats vacuum infiltrated with 20 μg/ml actinomycin D followed by 5 min Gro-lux then 2 min far red light (○) or vacuum infiltrated with 20 μg/ml actinomycin D followed by 5 min Gro-lux (□) or vacuum infiltrated with water followed by 5 min Gro-lux light (●) and (2) 4-day seedlings vacuum infiltrated with 20 μg/ml actinomycin D followed by 5 min Gro-lux illumination (△). These curves represent the average of two experimental replications.

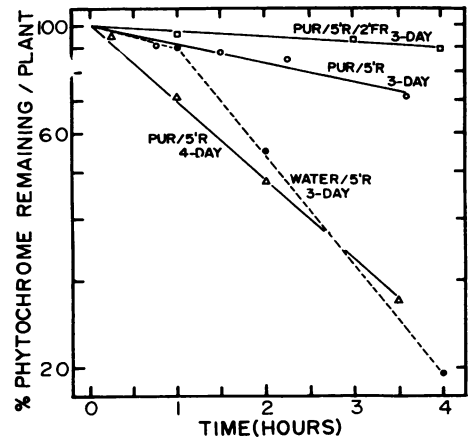

FIG. 5. Phytochrome destruction in (1) 3-day Garry oats vacuum infiltrated with 10 μg/ml puromycin followed by 5 min Gro-lux, then 2 min far red light (□) or vacuum infiltrated with 10 μg/ml puromycin followed by 5 min Gro-lux (○) or vacuum infiltrated with water, followed by 5 min Gro-lux light (●) and (2) 4-day seedlings vacuum infiltrated with 10 μg/ml puromycin followed by 5 min Gro-lux illumination (△). These curves represent the average of two experimental replications.

against the first hypothesis, thus suggesting that the second may be correct.

Since three commonly used inhibitors of protein synthesis prevent the appearance of the destruction mechanism (Figs. 3–5), we can conclude that the second hypothesis is, in fact, correct and that there is an apparent requirement for the occurrence of protein synthesis prior to the onset of destruction in young oat shoots. Apparently, the appearance of photoreversible phytochrome in a cell precedes the development of the ability of that cell to destroy Pfr. This requirement for protein synthesis to develop the capacity to destroy Pfr could be the result either of a direct involvement of an enzyme in carrying out the process or an indirect effect in that the proteins produced during the lag phase could in turn produce some other compound (such as the reported "Pfr killer" [6]) or structure which is required.

Two kinds of evidence support, although not exclusively, the interpretation that the lag phase is required for the synthesis of an enzyme which is directly involved in the destruction of Pfr. First, evidence is accumulating from measurements both *in situ* (12) and *in vitro* (Pratt and Kidd, manuscript in preparation) that material identifiable as phytochrome on the basis of recognition by specific antiphytochrome sera decreases in a parallel fashion with the loss of spectral photoreversibility. Second, factors which influence the destruction process, such as temperature, chelating agents, metal ions, and inhibitors, do so in a manner consistent with the hypothesis that phytochrome destruction involves an enzymatically mediated degradation of the chromoprotein (5, 8, 13).

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